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Plasma high density lipoprotein is increased in man when low density lipoprotein (LDL) is lowered by LDL-pheresis

(immunoadsorption/plasma exchange/familial hypercholesterolemia/atherosclerosis)

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ABSTRACT **Plasma high density lipoprotein (HDL) concentrations were increased in five hypercholesterolemic normoglyceridemic patients after removal of plasma low density lipoprotein (LDL) by LDL-pheresis. In each patient up to 80% of circulating LDL was removed by passing plasma through immunoadsorption columns containing antibody to apolipoprotein B immobilized to Sepharose. Rebound of LDL was slow after the procedure: 5–7 days in four non-familial hypercholesterolemic patients and >14 days in one patient with homozygous familial hypercholesterolemia. Plasma HDL rose above the pretreatment baseline during the interval between treatments in four of the five patients. When treatments were repeated weekly, time-averaged plasma LDL was lowered by 40–70%, while plasma HDL cholesterol and apolipoprotein AI were increased up to 2-fold, depending on the degree of LDL lowering. Plasma HDL concentrations fell back to their baseline values when LDL-pheresis was stopped and rose again when treatment was restarted. Thus, LDL-pheresis may augment the therapeutic effectiveness of LDL lowering by raising plasma HDL levels and the concentration of HDL relative to LDL.**

Homozygous familial hypercholesterolemia (HmFH) results from a variety of primary mutations, all affecting the gene that codes for the cellular low density lipoprotein (LDL) receptor (1). Patients with this disease are unable to clear LDL by LDL-receptor-mediated endocytosis and so have 5 times or more the normal concentration of LDL in their plasma. The extraordinarily high plasma LDL levels that occur in cases of HmFH are associated with early onset and rapid progression of atherosclerosis, often leading to death in the second decade of life (2). Thus, HmFH is a disease whose pathological consequences stem from the presence in plasma of a normal protein in abnormal amounts.

Whether as a result of high plasma LDL concentrations or for other reasons that are not understood, plasma concentrations of high density lipoprotein (HDL) are very low in patients with HmFH (3). HDL is thought to play an important role in plasma cholesterol transport and seems to protect against the progression of atherosclerosis (4): it accepts excess cholesterol from cells in culture (5–7) and helps to esterify plasma free cholesterol for reverse transport back to the liver (8–10). Moreover, plasma HDL concentrations are elevated at times when reverse transport of cholesterol is stimulated (11).

We know little of the role low plasma HDL concentrations may play in the early onset of atherosclerosis in HmFH, nor can we be sure that any benefit would be gained by normalizing HDL. However, if plasma HDL concentrations are lowered as a consequence of high plasma LDL levels in patients with hypercholesterolemia, then plasma HDL levels should rise when LDL is lowered. To test this possibility, we have measured plasma HDL concentrations before and after selective removal of plasma LDL from hypercholesterolemic patients by extracorporeal immunoadsorption (LDL-pheresis) (12, 13). Other factors known to affect plasma HDL were controlled by excluding hypertriglyceridemic patients and stopping the use of lipid-lowering drugs during the course of the study. Here we present evidence demonstrating that selective LDL-lowering by intensive LDL-pheresis raises plasma HDL.§

METHODS

Patients. The procedures used in these studies were approved by the Institutional Review Boards of Rockefeller University Hospital and New York Hospital–Cornell Medical College. Individuals were selected from a group of eight patients currently enrolled in a larger study of the safety and effectiveness of LDL-pheresis (14) by excluding those with plasma triglyceride levels above 200 mg/dl. Descriptive data for the five patients who met this criterion are shown in Table 1.

LDL-Pheresis. Immunoadsorption columns were made as described by Stoffel and his co-workers (12, 13), using monospecific polyclonal sheep anti-human LDL. LDL-pheresis was carried out essentially as described by Stoffel *et al.* (13) except for our use of an automatic column-regenerating unit and a modified procedure for initiating immunoadsorption that we have described previously (14).

Analytical Procedures. Cholesterol and triglycerides were measured by enzymatic procedures using commercial kits from Boehringer Mannheim. HDL was separated from very low density lipoprotein (VLDL) and LDL by precipitation with phosphotungstic acid (16) (patients A.F., K.K., and E.P.) or with dextran sulfate and $MgCl_2$ (17) (patients M.R. and P.S.). VLDL was calculated as the difference between total plasma and (LDL + HDL) cholesterol after flotation of VLDL (18). Determination of HDL by sequential flotation of lipoproteins was carried out in a Beckman L2-65B ultracentrifuge with a 40.3 rotor as described by Havel *et al.* (19). Apolipoproteins B, AI, and AII were determined by radioimmunoassay (20). Plasma protein was measured by the method of Lowry *et al.* (21) with crystalline bovine serum albumin (Sigma) as the reference standard.

Study Design. All patients received instruction in food selection and estimation of portion size from a nutritionist upon entry into the study and at 6-month intervals thereafter.

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Abbreviations: AI, AII, and B, apolipoproteins AI, AII, and B, respectively; VLDL, LDL, and HDL, very low, low, and high density lipoproteins, respectively; HmFH, homozygous familial hypercholesterolemia; PCA, portacaval anastomosis.

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Table 1. Clinical characteristics of patients

Patient	Age, yr	Sex	Wt,* kg	Plasma lipids,† mg/dl: Triglycerides	Plasma lipids,† mg/dl: Cholesterol	Diagnosis‡
E.P.	38	M	90	182 ± 57	223 ± 19	Ang, failed CABG
A.F.	56	M	69	148 ± 44	204 ± 20	Ang, diffuse CAD
M.R.	58	M	82	163 ± 3	268 ± 3	HC, Ang, CAD
P.S.	35	M	68	163 ± 50	305 ± 25	HtFH, Ang, CAD
K.K.	9	F	28	61 ± 11	568 ± 55	HmFH, AS, PCA

*All patients were within 5% of ideal body weight as determined from the Metropolitan Life Tables (15), except E.P. (115%), and body weights were maintained constant within ±5% over the course of the study.

†Data are presented as the mean ± SD (n = 3 except for patient K.K., for whom n = 5).

‡Ang, angina; CABG, coronary artery bypass graft; CAD, coronary artery disease confirmed by angiography; AS, aortic stenosis; HC, hypercholesterolemia; HtFH, heterozygous familial hypercholesterolemia; PCA, portacaval anastomosis.

Diet compositions were calculated from 9-day food records (22). The patients were maintained on a low-cholesterol (<250 mg/day) moderate-fat diet (30% of calories as fat of polyunsaturated-to-saturated weight ratio > 1), except for A.F. and M.R., who maintained themselves on a very-low-fat diet (<15% of calories, polyunsaturated-to-saturated ratio > 1). Baseline (basal) plasma lipids were measured three times or more during a 4-week initial diet period. Patients received ferrous sulfate by mouth to prevent iron deficiency.

The effect of LDL lowering on plasma LDL and HDL was studied through a series of treatment periods in which LDL-pheresis was carried out at various fixed intervals: 21, 14, 10, 7, or 3.5 days. Pre- and post-treatment samples were drawn at every visit. Data from the first two treatments in each period and any treatment deviating from the specified interval by more than ±2 days were excluded from analysis; fewer than 10% of all data were discarded for these reasons. After patients had reached a steady state as judged by the absence of any trend in the pre- and post-treatment lipid data, samples were collected daily to characterize the kinetics of lipoprotein rebound between LDL-pheresis procedures. Time-averaged plasma cholesterol and lipoprotein concentrations were calculated from these daily measurements.

Calculations and Statistics. Data are presented as the mean ± SD of n treatments. Differences between means were evaluated for statistical significance by a one-tailed Student's t test.

RESULTS

The mean pre- and post-treatment plasma lipoprotein concentrations measured in each treatment period are presented in Table 2. As was expected, the immunoadsorption columns effectively removed apolipoprotein-B-containing lipoproteins: VLDL, 47–85% and LDL, 54–82%.

Specificity. The selectivity of LDL-pheresis was evaluated by comparing removal of LDL to nonspecific removal of other plasma components. Small but significant reductions of plasma HDL concentration (Table 2) and total plasma protein (Table 3) were observed. An analysis of the waste washes and the fraction bound to the immunoadsorption columns during a representative LDL-pheresis in patient K.K. is shown in Table 3.

Apolipoprotein B, which is the core protein of VLDL and LDL, accounted for 81% of all protein bound to the immunoadsorption columns, fibrous or fibrin-like proteins for 10%, and other apolipoproteins for 6% of bound protein. Less than 4% of the bound protein was unidentified, and traces of albumin, IgG, IgA, IgE, and complement proteins were present in this fraction. Most (92%) of the cholesterol bound to the immunoadsorption columns was recovered as VLDL and LDL. Thus it appears that our immunoadsorption columns specifically bound VLDL and LDL. Although small quantities of other proteins were bound either directly to the matrix of the immunoadsorbant or by association to bound VLDL and LDL, this did not cause a significant degree of nonspecific removal of plasma proteins. Some of the nonspecific lowering of plasma protein and HDL during LDL-pheresis is due to dilution by excess saline infused during the procedure (13). After taking this into account the remaining losses can be accounted for as material lost to the void spaces of the extracorporeal system and collected in the waste washes (Table 3).

Table 2. Effect of LDL-pheresis on plasma lipoproteins

Patient	Treatment*: Days/no.	Treatment*: Interval, days	Sample	Lipoproteins,† mg/dl: VLDL	LDL	HDL
E.P.			Basal	59 ± 19	128 ± 16	36 ± 3
	230/23	10	Pre	23 ± 14¶	128 ± 10	38 ± 5
			Post	11 ± 12	54 ± 10	29 ± 5
A.F.			Basal	22 ± 4	152 ± 18	30 ± 3
	112/8	14	Pre	26 ± 14	141 ± 14	36 ± 3
			Post	6 ± 3	65 ± 11	25 ± 3
M.R.			Basal	28 ± 5	194 ± 9	46 ± 4
	119/17	7	Pre	31 ± 9	134 ± 34¶	50 ± 6
			Post	9 ± 5	62 ± 19	45 ± 6
	84/24	3.5	Pre	20 ± 9	111 ± 12¶	54 ± 9
			Post	3 ± 5	48 ± 10	47 ± 8
P.S.			Basal	29 ± 14	216 ± 14	60 ± 5
	91/13	7	Pre	30 ± 11	159 ± 19¶	73 ± 10§
			Post	9 ± 4	66 ± 35	55 ± 9
K.K.			Basal	46 ± 19	503 ± 44	19 ± 3
	189/9	21	Pre	23 ± 29§	372 ± 81¶	25 ± 4§
			Post	8 ± 10	168 ± 8	15 ± 3
	98/7	14	Pre	15 ± 14‡	364 ± 40¶	32 ± 3¶
			Post	8 ± 5	110 ± 31	17 ± 6
	42/6	7	Pre	15 ± 13§	238 ± 43¶	38 ± 7¶
			Post	4 ± 5	42 ± 23	27 ± 5

*Data shown are the length of the treatment period in days, the number of treatments carried out in the period, and the interval between treatments.

†Plasma lipoprotein concentrations were measured just before and immediately after each LDL-pheresis procedure. Results are presented as the mean ± SD for n equal to the number of treatments (column 2). Pre-treatment values were significantly different from basal values by Student's t test at the following levels: ‡, $P < 0.05$; §, $P < 0.01$; ¶, $P < 0.005$.

Table 3. Specific and nonspecific removal of plasma components during LDL-pheresis in patient KK

Fraction	Amount, g		
	Protein	Cholesterol	
Removed from plasma*	11.2	3.02	
Recovered in waste†	9.1	Trace	
Desorbed from immunoadsorbent‡	2.2	3.00	
Apolipoproteins		VLDL§	9 ± 4%
B	1.78	LDL§	82 ± 4%
AI	0.053	HDL§	7 ± 4%
E	0.034	Bottom§	2%
CIII	0.036		
Fibrin	0.220		
Other¶	0.077		

*The amounts of plasma protein and cholesterol removed from patient K.K. were calculated as the difference between pre- and post-treatment plasma concentrations after correction for dilution. It was assumed that the amount of additional saline returned during LDL-pheresis was equal to the change in body weight divided by the density of saline (1.006 g/ml) and that this excess was distributed in the total extracellular space (20% of body weight).

†All fluid not returned to the patient (exclusive of cellular elements).

‡The immunoadsorption columns used to treat patient K.K. were washed with 5 liters of saline and the bound material was desorbed with acid glycine buffer and brought to pH 7.0. A fibrous precipitate and clear supernatant were separated by low-speed centrifugation and then analyzed for proteins and cholesterol.

§Desorbed lipoproteins were separated by sequential flotation in the ultracentrifuge. Results are expressed as percent of total cholesterol (mean ± SD of four determinations).

¶Traces of IgG, IgA, and complement component 3 were detected by radial immunodiffusion.

Short-Term Changes in Plasma Lipoproteins After LDL-Pheresis. Short-term changes in plasma lipoprotein concentrations between LDL-pheresis treatments are shown in Fig. 1 for patients K.K. (treatment at 14-day intervals) and M.R. (treatment at 7-day intervals). VLDL concentrations returned to their pretreatment levels in the first day or two after treatment. LDL concentrations rebounded more slowly and reached pretreatment levels within 10–14 days in K.K. and within 5–10 days in M.R. Plasma HDL levels rebounded from their post-treatment levels faster than LDL and rose above pretreatment levels in the two studies shown in Fig. 1 and in four of the five patients studied (data not shown).

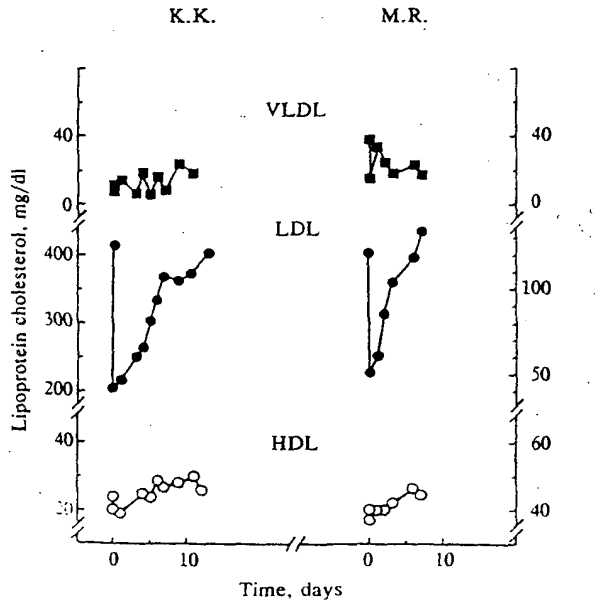

FIG. 1. Short-term changes in plasma VLDL, LDL, and HDL cholesterol concentrations after LDL-pheresis.

Longer-Term Changes. Low plasma LDL concentrations were maintained by repeating LDL-pheresis at frequent intervals. This is illustrated in Fig. 2 by a series of seven weekly treatments carried out in patient K.K. With each successive LDL-pheresis, plasma LDL concentrations fell lower, until a new balance was achieved between removal and synthesis. Increases in plasma HDL concentrations were clearly demonstrable after the fifth procedure, at day 26.

This effect was noticed again in another series of LDL-phereses carried out every 2 weeks in patient K.K. (Fig. 3*A*); it could be reversed by stopping LDL-pheresis and allowing plasma LDL concentrations to rise (Fig. 3*B*). In this study, plasma apolipoprotein AI concentrations were found to vary in parallel with HDL cholesterol. During LDL-pheresis (pretreatment data) HDL concentrations were 40 ± 3 and AI, 136 ± 19 mg/dl; and after LDL-pheresis had been stopped, HDL averaged 21 ± 2 and AI, 87 ± 8 mg/dl. Three methods used to quantitate HDL yielded changes in HDL of similar magnitude.

Relationship Between LDL Lowering and Increased HDL. HmFH is a rare genetic disorder and patient K.K., who had been subjected to portacaval anastomosis (PCA) 6 months before the present study, may have been unique. PCA generally lowers LDL by 15–30% in HmFH but has little or

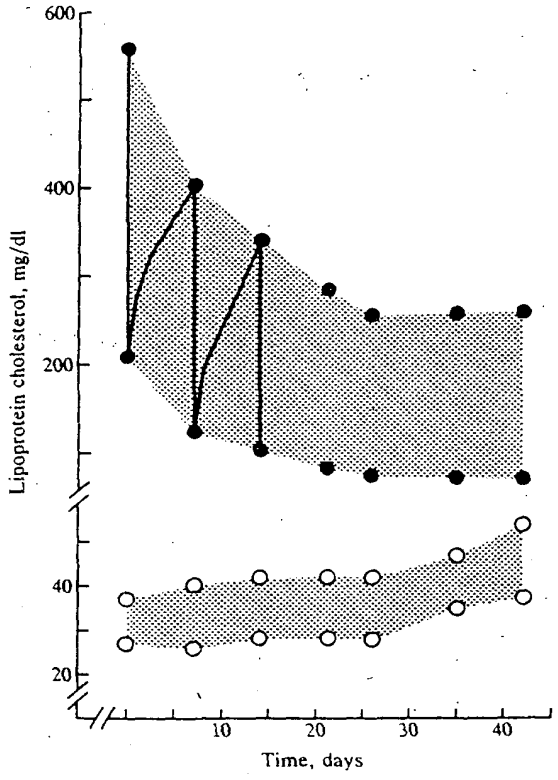

FIG. 2. Effect of repeated LDL removal by LDL-pheresis on plasma LDL and HDL concentrations in a patient with HmFH (K.K.). The pre- and post-treatment LDL (●) and HDL (○) concentrations are shown for a series of seven treatments carried out at intervals of 7, 7, 7, 5, 9, and 7 days.

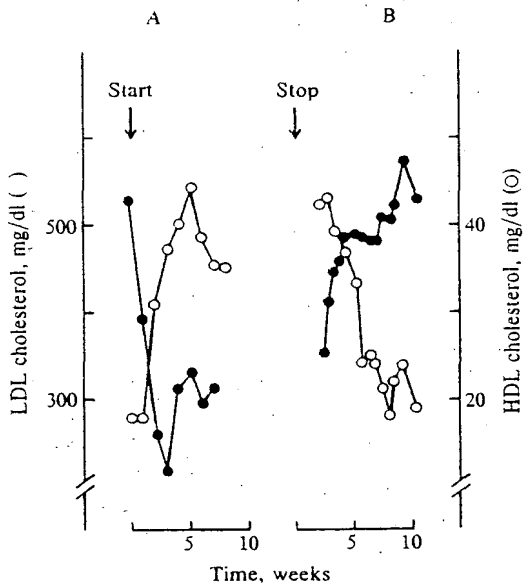

FIG. 3. Temporal relationship between LDL depletion and plasma HDL concentration in a patient with HmFH (K.K.). (*A*) Pretreatment LDL (●) and HDL (○) concentrations are shown for a series of LDL-pheresis treatments carried out weekly for 8 weeks. (*B*) Plasma LDL and HDL concentrations over a 10-week period after LDL-pheresis had been stopped.

no effect on plasma HDL (3). In patient K.K., pre- vs. post-PCA LDL and HDL were 683 ± 58 and 19 ± 2 mg/dl vs. 503 ± 44 and 19 ± 3 mg/dl, respectively, suggesting no special effect of PCA on HDL levels despite significant LDL lowering. Only when plasma LDL concentrations were brought below 400 mg/dl in patient K.K. by LDL-pheresis were her plasma HDL levels increased. To determine whether LDL-lowering raised HDL in other patients with hypercholesterolemia, we plotted the increments in plasma HDL as a function of the decrease in plasma LDL achieved, averaging the data obtained in each treatment period (Fig. 4). The result revealed a trend toward higher HDL concentrations as LDL levels were lowered in this small group of patients. Nevertheless, these data remain too preliminary to support extensive generalization of this trend to other populations.

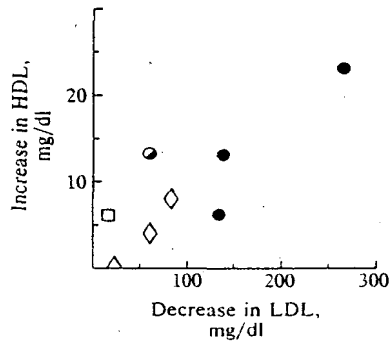

FIG. 4. Relationship between LDL lowering and the increase in plasma HDL in five normoglyceridemic patients with hypercholesterolemia. The increase (expressed as the mean of pretreatment values from each treatment period minus baseline) in plasma HDL is plotted against the decrease in LDL (data from Tables 1 and 2). △, E.P.; □, A.F.; ◇, M.R.; ◑, P.S.; and ●, K.K.

DISCUSSION

Lowering LDL Raises HDL. Transient elevations of HDL apoprotein above baseline have been reported after plasma exchange (23), but net increases of plasma HDL were not observed. Affinity adsorption by means of heparin-Sepharose columns was shown to raise plasma HDL in two patients with HmFH (24). But these changes in HDL could not be clearly related to the lowering of LDL because the patients also received clofibrate, a drug known to alter the metabolism of VLDL and to raise HDL (25). The patients described in this report received no lipid-lowering drug. So the three factors we believe most likely to have affected HDL metabolism in our patients are (*i*) the use of heparin as an anticoagulant, (*ii*) the periodic removal of a small fraction of the HDL pool with each LDL-pheresis treatment, and (*iii*) the change in LDL-to-HDL ratio imposed by repetitive LDL-pheresis.

Intravenous administration of heparin displaces lipoprotein lipase from the capillary endothelium and causes a transient increase in the rate of lipolysis and transfer of apolipoproteins and cholesterol from VLDL to HDL (26); thus weekly injections of heparin might increase lipoprotein lipase production and thereby cause a lasting increase in plasma HDL. But this has not been observed in patients undergoing long-term plasmapheresis (27). Moreover, when heparin was replaced by citrate (patients K.K. and E.P.), HDL concentrations remained elevated as long as treatment was continued (up to 6 months).

Patients undergoing frequent LDL-pheresis are subject to periodic removal of about 5% of the total HDL apolipoprotein (vascular + extravascular). This might be sufficient to stimulate HDL synthesis and thus lead to elevated plasma HDL concentrations.

Finally, the relatively high ratio of LDL to HDL concentrations might alter competition between LDL and HDL for exchangeable apolipoproteins or for binding to catalytic factors (plasma lecithin:cholesterol acyltransferase, lipoprotein lipase, hepatic lipase, or lipid transfer proteins) so as to lower plasma HDL concentrations in hypercholesterolemia. In this case LDL-pheresis might increase plasma HDL concentrations by restoring the LDL-to-HDL ratios to more normal values.

Therapeutic Considerations. Recent studies of atherosclerosis progression in man and regression in animals have emphasized the importance of various ratios of LDL to HDL cholesterol or total plasma cholesterol to HDL as predictors of risk. For example, regression of atherosclerosis in nonhuman primates has been observed in animals in which the ratio of total to HDL cholesterol was less than 4.5 (28).

Weekly LDL-pheresis of K.K. (the patient with HmFH) brought the ratio of total to HDL cholesterol from a basal level of 30 to 5.2 (mean of pre- and post-treatment data). Weekly LDL-pheresis of non-familial hypercholesterolemia patients resulted in ratios (calculated as the mean of daily measurements) of 3.34 and 3.0 in patients M.R. and P.S., respectively, and twice-per-week treatment of M.R. lowered the ratio further to 2.6. Net increases in HDL contributed to these changes in the ratios. Although the mechanism by which LDL-pheresis raises HDL remains unclear, we were able by these means to effectively control hyperlipidemia in our patients and to maintain conditions that have been shown to promote reverse cholesterol transport and regression of atherosclerosis in animals.

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